

WHAT IS CLAIMED IS:

We claim:

1. A method of synthesizing a mammalian, essentially pure, glycosylated Dkk protein comprising the steps of:
 - (a) harvesting culture media from a mammalian cell line with a nucleic acid encoding a mammalian Dkk protein in a replicating vector that synthesizes Dkk protein, wherein said Dkk protein comprises a signal peptide for expression and secretion into media of said Dkk protein;
 - (b) purifying the filtered culture media across an affinity gradient column;
 - (c) collecting Dkk protein containing fractions from the column;
 - (d) concentrating the Dkk protein containing fractions in a phosphate buffered saline in the presence of a detergent and EDTA to produce a concentrated, mammalian, essentially pure, glycosylated Dkk protein.
2. The method of claim 1, wherein the method further comprises purifying the Dkk protein using preparative and analytical size-exclusion chromatography.
3. The method of claim 1, further comprising treating the culture media with one or more protease inhibitors.
4. The method of claim 1, further comprising the step of filtering the culture media prior to purifying the culture media.
5. The method of claim 1, wherein the detergent is Tween, CHAPS, N-octyl- β -D-glucoside, triton X-100, or Nonidet P40.
6. The method of claim 1, wherein the affinity column is a metal affinity column.

- 44 -

7. The method of claim 1, wherein the size exclusion column is a Superose-12 column, a Superdex-200 column, a Sephacryl column, or a Sephadex column.
8. The method of claim 6, wherein the metal is nickel, zinc, or iron.
9. The method of claim 5, wherein the Tween is Tween-20 in the amount of about 0.01% to about 1% Tween-20 and EDTA is present in the amount of about 0.01 mM to about 2 mM EDTA.
10. The method of claim 5, wherein the Tween-20 is present from about 0.005% to about 0.1% or N-octyl- β -D-glucoside from 0.05 to 0.7% and the EDTA is present in the amount of about 0.5 mM EDTA.
11. The method of claim 5, wherein the N-octyl- β -D-glucoside is present in the amount from about 0.05% to about 0.7% and EDTA is present in the amount of about 0.5M.
12. The method of claim 9, further comprising the step of lyophilizing the essentially purified Dkk protein.
13. The method of claim 1, wherein the Dkk protein is Dkk1.
14. The method of claim 13, wherein the Dkk1 protein is human Dkk1.
15. The method of claim 3, wherein the treating step is additionally performed in the presence of a salt and imidazole.
16. The method of claim 15, wherein the salt is NaCl, LiCl, or KCl, and wherein the salt is in a final concentration of about 100 mM to about 1 M, and the imidazole is present in a final concentration of about 0.5 mM to about 50 mM imidazole.
17. The method of claim 15, wherein the salt is NaCl and is present at a final concentration of about 500 mM, and the imidazole is present at a final concentration of about 5 mM.

18. The method of claim 1, wherein the affinity gradient is an imidazole gradient of about 5 to about 1,500 mM imidazole in a metal column, and wherein the Dkk protein is tagged with histidine.

19. The method of claim 18, wherein the imidazole gradient is about a 20 mM to about a 1,000 mM imidazole gradient.

20. The method of claim 18, wherein the Dkk protein is human Dkk1, and the metal column is a nickel column.

21. The method of claim 1, wherein the mammalian cell line is HEK293T cells or HEK293 EPNA cells.

22. A purified, glycosylated Dkk protein produced by the method of claim 1.

23. The purified, glycosylated Dkk protein of claim 22, wherein the essentially purified, glycosylated Dkk1 protein is a human Dkk1 protein.

24. The purified, glycosylated Dkk protein of claim 22, wherein the Dkk protein further comprises a selectable tag.

25. The purified, glycosylated Dkk protein of claim 24, wherein the selectable tag is a *c-myc* tag or a His₆-tag.

26. The purified, glycosylated Dkk protein of claim 25, wherein the selectable tag is a His₆-tag.

27. The purified, glycosylated Dkk protein of any of claims 18 or 19, wherein the Dkk protein further comprises a proteolytic cleavage site between the selectable tag and the Dkk protein.

28. A purified, glycosylated, mammalian Dkk1 protein having at least one of the following properties:

- 46 -

- (a) a molecular weight of approximately 40 kD \pm 2.0 kD as determined by SDS-PAGE;
- (b) inhibits Wnt3A activity; and
- (c) co-immunoprecipitates a LRP5 protein or a fragment thereof comprising the ligand binding domain.

29. A purified, glycosylated, mammalian Dkk1 protein having at least one of the following properties:

- (a) a molecular mass of about 36.1 kD to about 36.8 kD as determined by ESI-MS;
- (b) a weight average molar mass of about 36 to about 46 kD of a Dkk1
- (c) an S-value of 2.8 for monomeric Dkk1 protein as determined by AUC analysis;
- (d) a weight average molecular mass of about 74 kD of a Dkk1 homodimer as determined by SEC-MALLS;
- (e) a Dkk1-LRP5 binding stoichiometry of 1:1 as determined by AUC and/or SEC-MALLS;
- (f) a molar extinction coefficient of $1.96 \times 10^4 \cdot \text{M}^{-1} \cdot \text{cm}^{-1}$ as determined based on the amino acid sequence of Dkk1 and changes in refractive index of Dkk1 due to glycosylation as measured using a high performance liquid chromatography refractometer;
- (g) a change in specific refractive index increment of Dkk1 due to glycosylation as measured using a differential refractometer; and/or
- (h) a dn/dc value of 0.186 for an unmodified Dkk1 protein and a dn/dc value of 0.130 to 0.180 of glycosylated protein due to post translational modifications.

30. The purified protein of claim 29, wherein the Dkk1 protein is human Dkk1 protein.

31. The purified protein of claim 29, wherein the protein comprises a His tag.

- 47 -

32. The purified, glycosylated Dkk protein of claim 24, wherein the selectable tag is a *c-myc* tag.

33. The purified protein of any of claims 31 or 32, wherein the Dkk protein further comprises a proteolytic cleavage site between the tag and the Dkk protein.

34. The purified protein of any of claims 28 or 29, wherein the protein inhibits Wnt3a activity by at least about 50%.